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Original communication

Does β-APP staining of the brain in infant bed-sharing deaths differentiate these cases from sudden infant death syndrome?☆

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ABSTRACT

Archival cerebral tissue from infants whose deaths were attributed to sudden infant death syndrome (SIDS) from South Australia and Western Denmark were stained for β-amyloid precursor protein (β-APP) and graded according to a simple scoring chart. The resulting APP scores were correlated with sleeping situation (shared vs. alone) showing a significantly higher amount of β-APP staining in the non-bed-sharing, than in the bed-sharing infants (Mann–Whitney, Australia: $p = 0.0128$, Denmark: $p = 0.0014$, Combined: $p = 0.0031$). There was also a marked but non-significant difference in sex distribution between bed-sharers and non-bed-sharers with a male to female ratio of 1:1 in the first group and 2:1 in the latter. Of 48 Australian and 76 Danish SIDS infants, β-APP staining was present in 116 (94%) cases. The eight negative cases were all from the Danish cohort. This study has shown that the amount of β-APP staining was significantly higher in infants who were sleeping alone compared to those who were bed-sharing with one or more adults, in both an Australian and Danish cohort of SIDS infants. Whether this results from differences in the speed with which these infants die, differences in lethal mechanisms involving possible accidental asphyxiation in shared sleepers, or differences in the number of previous hypoxic-ischemic events, remains to be clarified.

1. Introduction

Unexpected infant deaths may result from a wide variety of causes that include trauma and identifiable organic diseases.[1,2] In other situations the diagnosis is more difficult to make due to a lack of pathognomonic features at autopsy. Typically these cases include sudden infant death syndrome (SIDS) and accidental or deliberate asphyxiation.[3] It is now recognized that the diagnosis of asphyxia at all ages is problematic due to a lack of diagnostic pathological features.[4,5] This situation is even more difficult in cases of infant deaths in shared sleeping situations where airway compromise may occur without leaving any markers.[6] The literature is divided as to the cause of these deaths, with a number of authors attributing all of these deaths to SIDS.[7–10] It is, however, difficult to understand how the possibility of accidental asphyxia can be so confidently excluded.[3,11–14]

β-amyloid precursor protein (β-APP) is a membrane glycoprotein found in neurons that significantly up-regulates after injury and accumulates within axons.[15–17] We have been studying β-APP in the brains of SIDS and other infants and have identified cases where multifocal deposition is present.[18,19]

A recent study by the authors demonstrated a significantly different sex ratio in infants who died in a shared sleeping situation compared to those who had died alone in their cribs.[20] The alone sleepers had the typical increased male to female ratio that characterizes most SIDS studies, contrasting markedly with the shared sleepers whose sex ratio approached unity.[6,21] This raised the possibility that the two populations were different. To investigate this further we decided to examine the intensity and amount of β-APP staining in the brains of infants who had died alone, and compare it to those who died while sharing a sleeping

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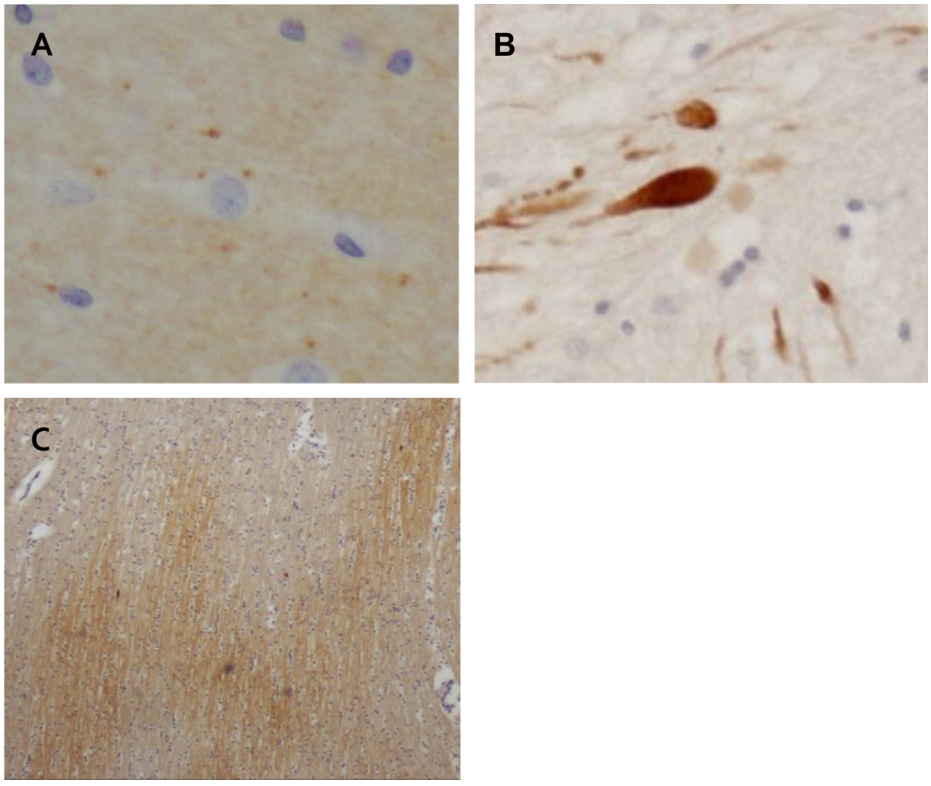

Fig. 1. Immunohistochemical staining for β-APP demonstrating a variety of patterns: (A) granular change (400× magnification), (B) swellings (200× magnification), (C) bands (40× magnification).

surface, using a recently developed scoring system for β-APP staining.[19]

2. Materials and methods

All cases of SIDS from South Australia (population 1.6 million) autopsied at Forensic Science SA (FSSA) over a seven-and-a-half-year period (mid 1999–2006), and all SIDS cases from the Western part of Denmark (population 2.3 million) over a 16-year period (1992–2007) autopsied at the Department of Forensic Medicine, Aarhus University were accessioned. The diagnosis of SIDS was made according to the San Diego criteria.[22,23] Case files including police reports and hospital records were reviewed and relevant data entered into a database using EpiData version 3.1 software.

Sections were stained for β-APP according to standard methodology and graded according to a newly developed and statistically validated scoring method.[19] In summary, β-APP positive axonal changes were divided into patterns of granular change, swellings, retraction bulbs and bands (Fig. 1). Stained sections from eight anatomical regions were examined, including the corpus callosum, internal capsule, hemispheric white matter (internal capsule excluded), cerebellum, midbrain, pons, medulla oblongata and spinal cord. There was a maximum score of nine (3 − granular changes; 3 − swellings, 1 − retraction bulbs, and 2 − bands) for the corpus callosum, internal capsule, hemispheric white matter (internal capsule excluded), cerebellum, and a score of 8 for the brainstem and spinal cord (retraction bulb scoring was not included as many brainstem and spinal cord axons travel in the longitudinal plane making it difficult to assess retraction bulbs on transverse sections). The results were expressed as a percentage of the maximum possible for each anatomical region and for the regions combined. The grading was blinded, and all sections were scored by the same author (LLJE).

3. Results

There were 48 infants from South Australia and 76 infants from Western Denmark. One Australian case was excluded as there was only one section available and one Danish case was excluded as it had not been possible to locate any of the tissue blocks. A total of 1301 Australian and 645 Danish sections were examined ($N = 1946$). Shared sleeping (bed sharing) was defined as an infant sharing a surface such as a bed, mattress or couch with one or more adults during sleep.

Bed sharing was found in similar proportions in the Australian and Danish cases (Australia 31%; Denmark 28%) (Table 1). The mean β-APP score in the Australian cases of 28 (range 5–67) was significantly higher than the mean score of 10 (range 0–35) in the Danish cases (Mann–Whitney, $p < 0.0001$). For this reason, analyses were initially conducted for each country separately. β-APP staining was found in 94% of the cases. The eight β-APP-negative cases were all from the Danish cohort. The amount of β-APP was significantly higher in the non-bed-sharing infants than in the bed-sharing

Table 1
Data on sleeping arrangements for infants in Australia and Denmark.

	Country		Total (%)
	Australia (%)	Denmark (%)	
Bed-sharing	15 (31)	21 (28)	36 (29)
Non-bed-sharing	32 (67)	52 (68)	84 (68)
Missing information	1 (2)	3 (4)	4 (3)

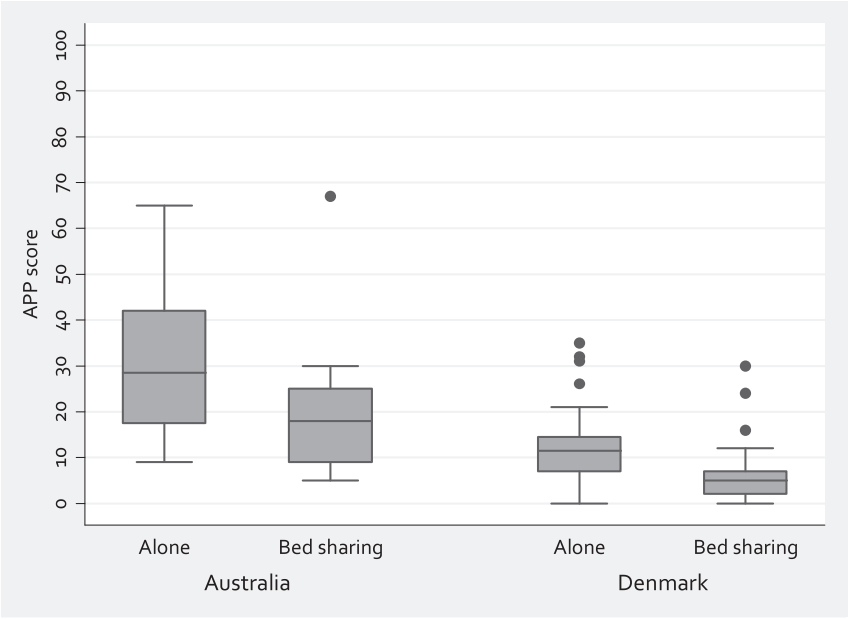

Fig. 2. β-APP scores in relation to bed sharing status and nationality. The differences between the β-APP scores of the bed-sharing and non-bed-sharing infants were significant for both countries (Mann–Whitney, AUS: $p = 0.0128$, DK: $p = 0.0014$, combined: $p = 0.0031$).

infants in both groups (Mann–Whitney, AUS: $p = 0.0128$, DK: $p = 0.0014$, combined: $p = 0.0031$) (Fig. 2).

Although there was a trend for a male predominance in the non-bed-sharing group (male: 65%, female: 35%; M:F = 2:1) compared to an equal sex distribution (male: 50%, female: 50%; M:F = 1:1) in the bed-sharing group, this did not reach significance (Fisher's exact test, $p = 0.153$).

Infants were found prone in 23 (48%), supine in 17 (35%) and on the side in 4 (8%) of the Australian cases. In the Danish cases 30 (39%) were found prone, 14 (18%) supine, and 12 (16%) on the side. The differences between these two cohorts were not statistically significant (Fisher's exact test, $p = 0.156$). The amount of APP did not differ significantly between the three sleeping position groups. Information on sleeping position was absent in 4 (8%) of the Australian cases and in 20 (26%) of the Danish cases. The infants' faces were covered in 33% of the Australian and in 24% of the Danish cases, but this was not significant (Pearson chi-squared, $p = 0.295$). Face-covering was not correlated with β-APP staining in either of the two cohorts. No differences were found in the amount of β- APP staining relating to mean age, sex and gestational age.

4. Discussion

Attempting to differentiate overlaying deaths from SIDS has always been a contentious exercise. While there is no doubt that not all shared sleeping situations are dangerous, it seems reasonable to accept that some may be unsafe.[13] For example, most would advise physically large parents who are tired and/or intoxicated, and who are lying on a soft surface, against sleeping with their infant.[24–26] Unfortunately the autopsy findings in cases where there are fatalities are usually identical to those of SIDS, and so differentiating these two potential types of deaths on pathological grounds is not possible.[5,6] The current study has, however, looked at a marker of axonal injury that may reflect prolonged cerebral hypoxic-ischemia in infants who died alone in their cribs compared to those who died while sharing a sleeping surface.

The difference in the sex ratio of the alone and shared sleeping Australian infants was the subject of a previous report, generating the hypothesis that the difference between the two groups suggests that they may represent different populations.[20] It was of interest that the Danish shared sleeping group in the present study also showed a similar sex ratio of unity, contrasting again with the alone sleepers, although the lower numbers did not reach significance. It would be useful to have sex ratios reported from larger studies where full death scene examinations have been performed.

The increased amount of β-APP staining in the Australian group may have been influenced by differences in staining techniques and also by variations in areas of tissue sampling. However, despite these differences, the same statistically significant findings were demonstrated in both groups.

As is often the case in studies of SIDS and other causes of unexpected and sudden infant death there may be considerable overlap between cases that precludes using a particular feature as a diagnostic sign. However, while differences in β-APP staining between alone and shared sleepers may not enable definitive separation, it does show us that these groups appear, for whatever reason, to be different.

In summary, this study has shown that another population of shared infant sleepers has had a trend for relatively less male deaths, in keeping with the proposition that there may be differences between these two groups. Of additional interest is the fact that cerebral β-APP staining in shared sleeping infants was statistically less than that of alone sleeping infants. While the increased amounts of β-APP in alone sleepers may suggest previous hypoxic-ischemic episodes, it may also reflect a slower anoxic mode of death which has allowed β-APP to accumulate agonally, as β-APP may be observed within 35 min of a cerebral insult.[27,28] Thus, if the deaths in shared sleeping environments are occurring more rapidly due to suffocation from overlaying, it would not be unexpected that there would be less β-APP staining. While it is possible to conjecture as to the reason for these results, this study of β-APP staining of the brain has demonstrated that infants who die in shared, compared to an alone, sleeping situation do have distinct differences that require explanation.

Ethical approval
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Conflict of interest
None declared.

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